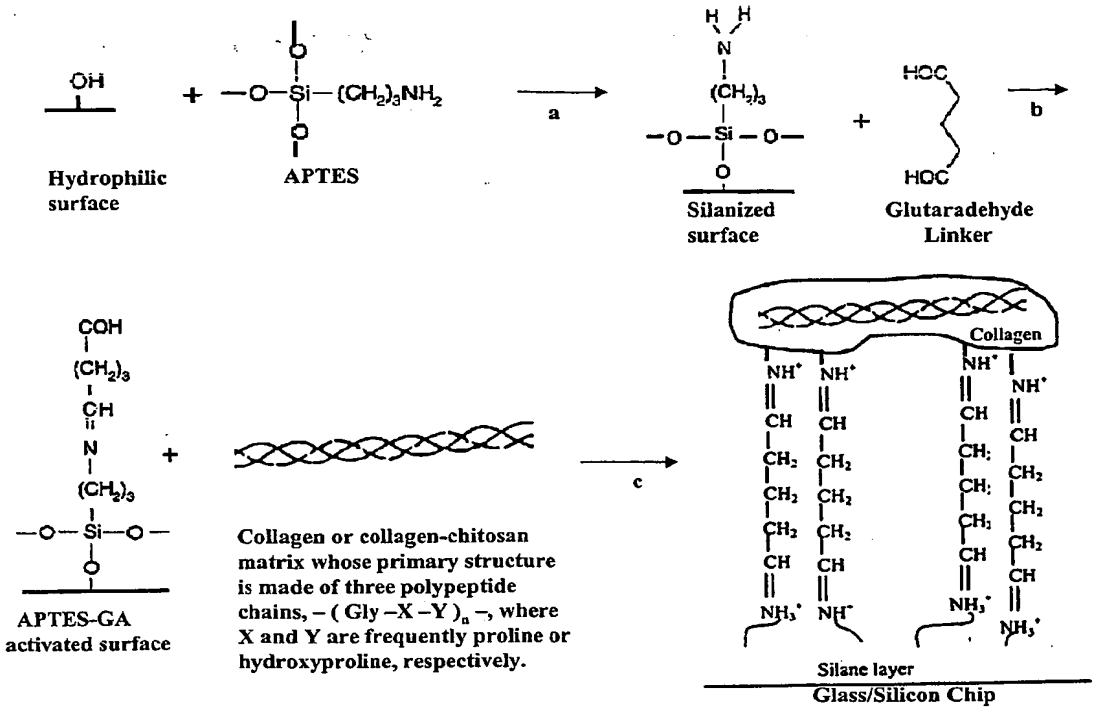

Fig 1. Surface modification procedure involved in cell-collagen gel immobilization. (a) Silanize surface with APTES; (b) Crosslink surface with glutaraldehyde; (c) Add cell-collagen matrix.

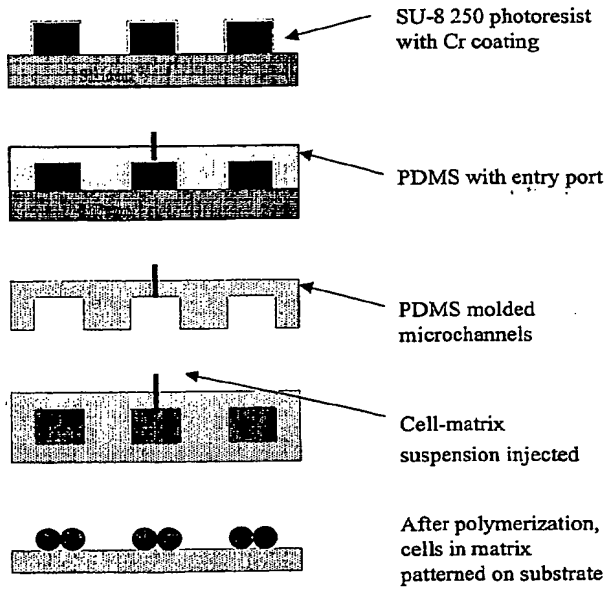

Fig 2. Flow chart of preparing cell patterns on the substrate using microfabrication and microfluidic techniques

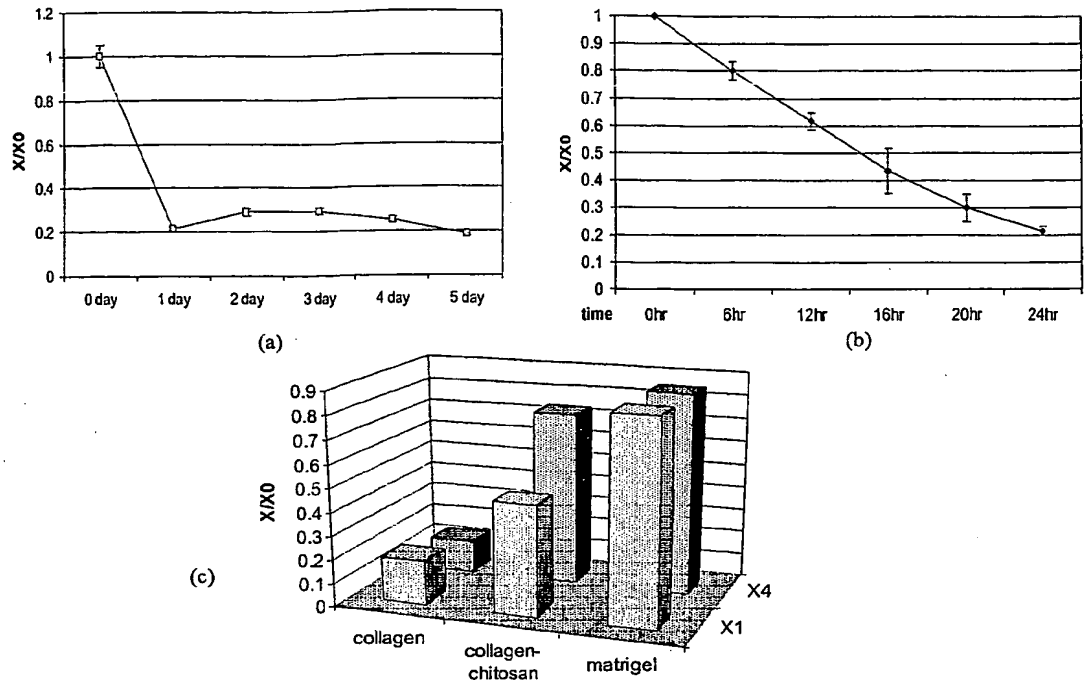

Fig 3. The contraction of collagen matrix (0.8mg/ml) with cell density of 3X10^{5}cells/ml. (a) Contraction by fibroblasts over days; (b) contraction by fibroblasts over hours; (c) contraction of different matrices by SMC. (Those in X4 has four times of cell density than those in X1)

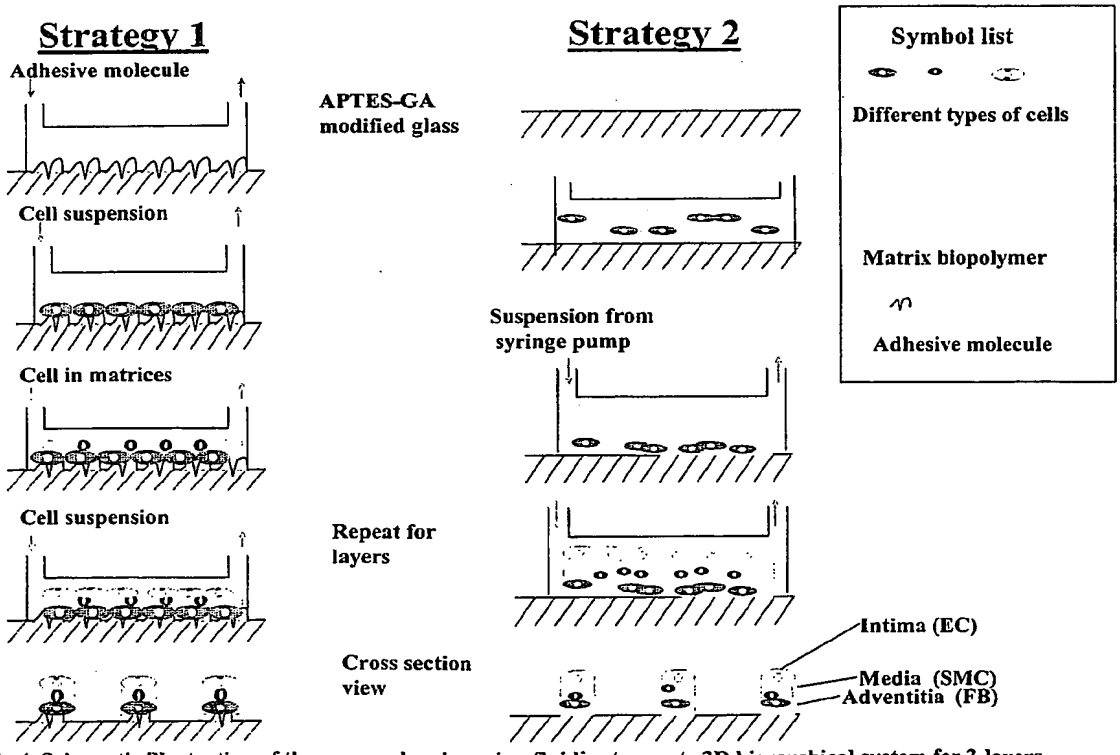

Fig 4. Schematic illustration of the approach using microfluidics to create 3D hierarchical system for 3-layers of cells and biopolymer matrices.

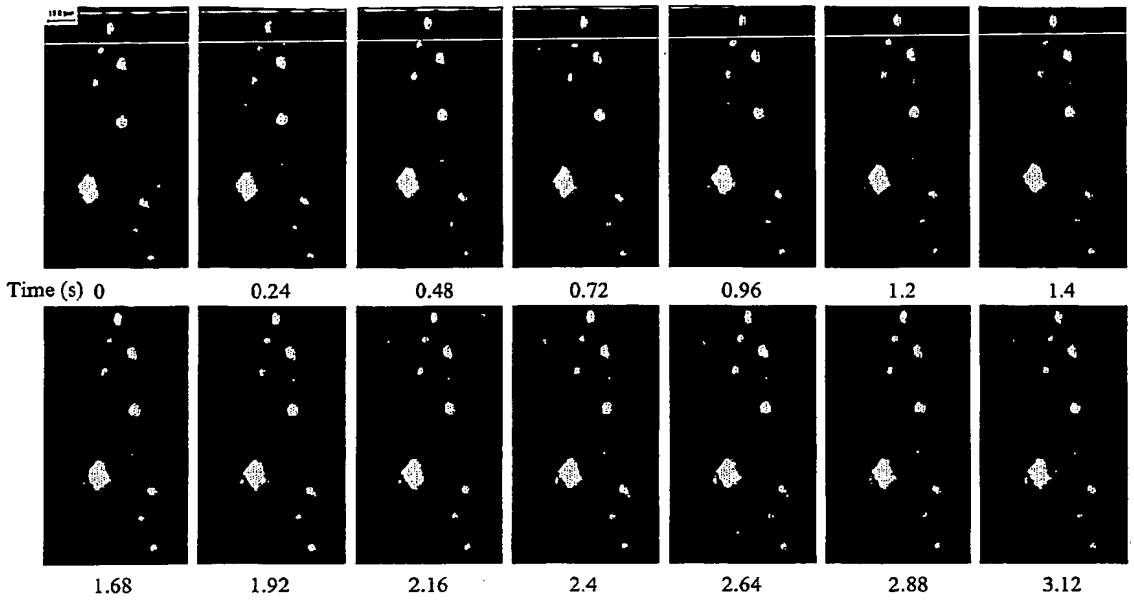

Fig 5. Time-lapse video image sequences of bottom layer under shear stress of fluidic delivery of a new layer. The displacement of the bottom fibroblast layer in the matrix is observed.

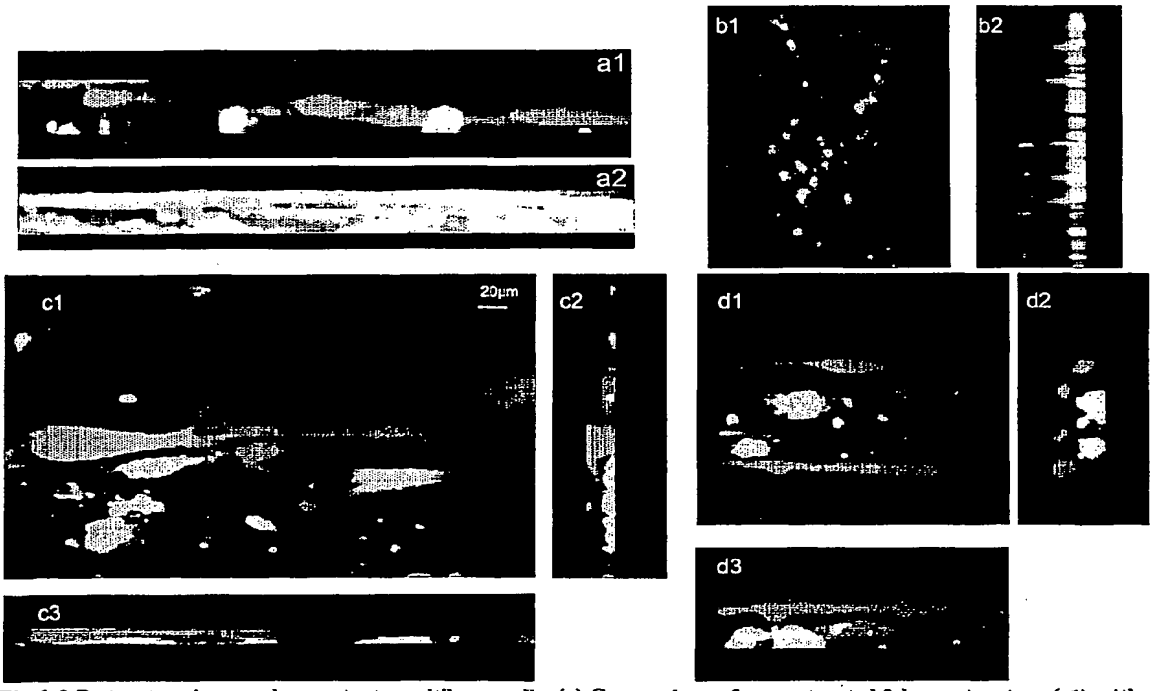

Fig 6. 3-D structure images demonstrate multilayer cells. (a) Comparison of reconstructed 2-layer structure (a1) with mixed 3-D coculture(a2); (b) 3-D images of a 3-layered structure of three cell types with angle of view at 15° (b1) and 90° (b2); (c) Images of reconstructed 3-D stacks of a 2-layer structure using strategy1(c) and strategy2(d) with SMCs (green) on top and fibroblasts(red) at the bottom (c1 and d1 are images rotating vertically 0, c2 and d2 90°, c3 and d3

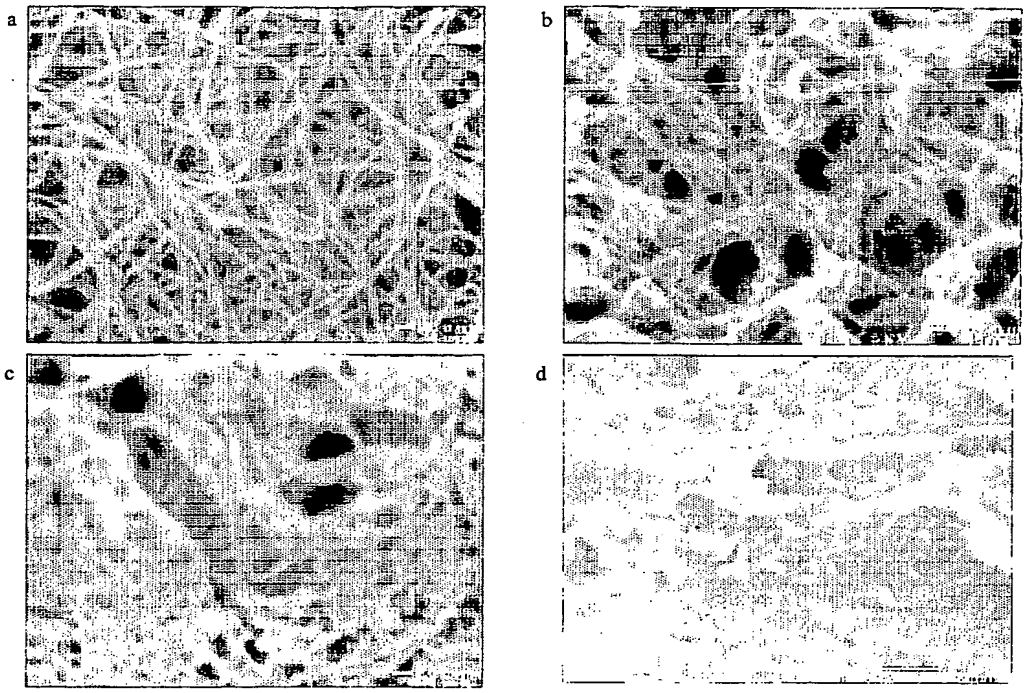

Fig 7. SEM micrographs show the ultrastructure of different matrices: (a) Pure collagen matrices; (b) Collagen-chitosan matrices with proportion of 1:1; (c) Collagen-chitosan matrices with proportion of 1:3; (d) Matrigel matrix. (Collagen concentration is 0.8mg/ml)

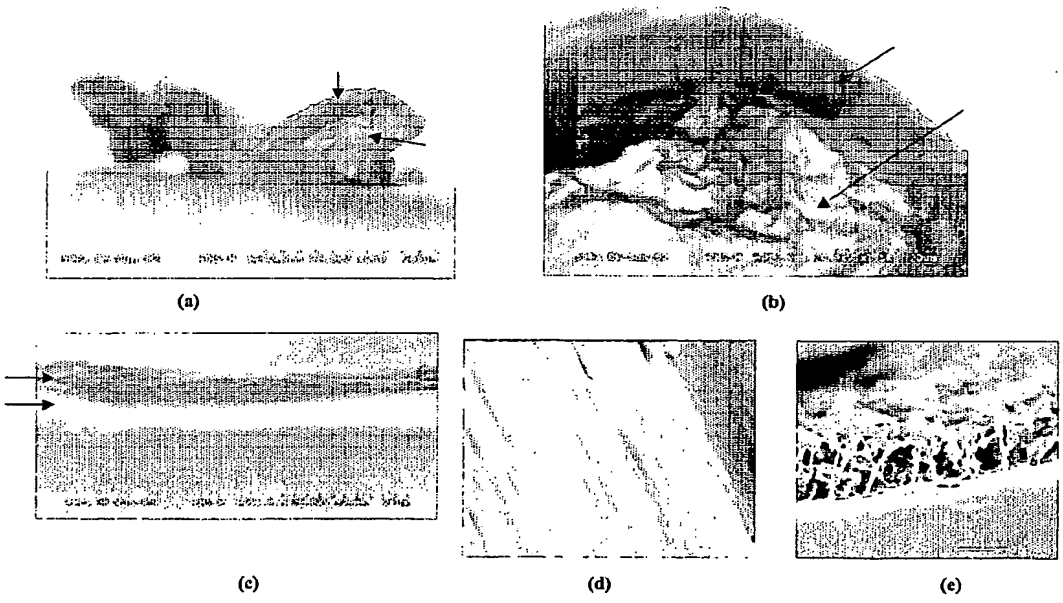

Fig 8. SEM micrograph of the two-layer structure of the model from one end of the pattern (a-b) and from the side view of it (c). The arrows are pointed to the two different fiber structures. The bottom layer is fibroblasts in collagen matrix (d) and the top layer is collagen-chitosan matrix (e)

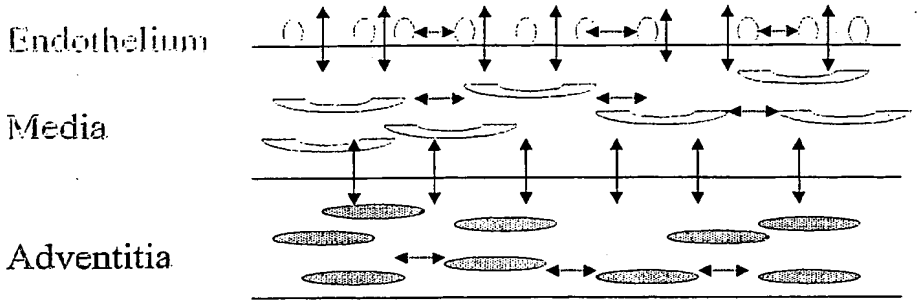

Fig 9. Biomimetic cellular interaction paradigm: Different cell types interact with each other between layers, but the cells of same types interact with each other on the same layer. The arrows represent the cell-cell interaction between layers and within a layer.

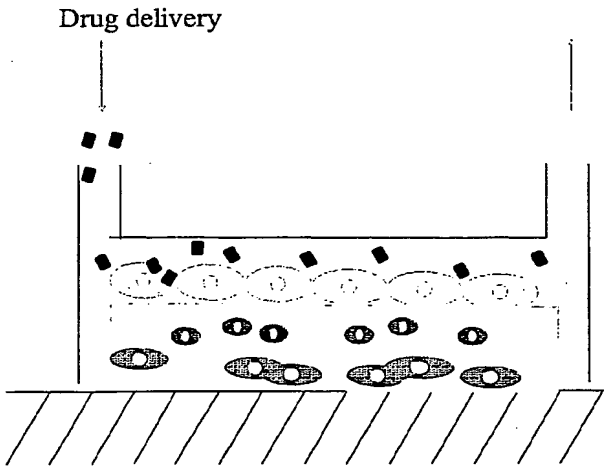

Fig 10. Schematic illustration of using the bio-mimetic layer structures for drug screening model

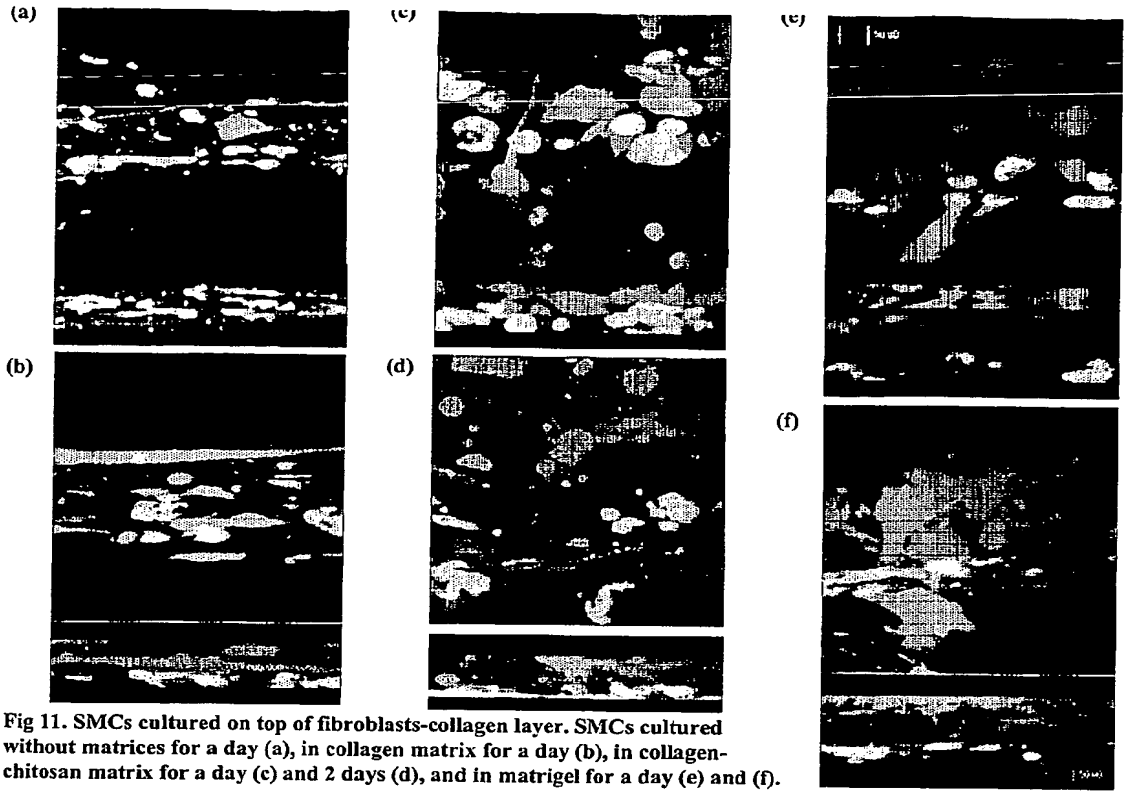

Fig 11. SMCs cultured on top of fibroblasts-collagen layer. SMCs cultured without matrices for a day (a), in collagen matrix for a day (b), in collagen-chitosan matrix for a day (c) and 2 days (d), and in matrigel for a day (e) and (f).

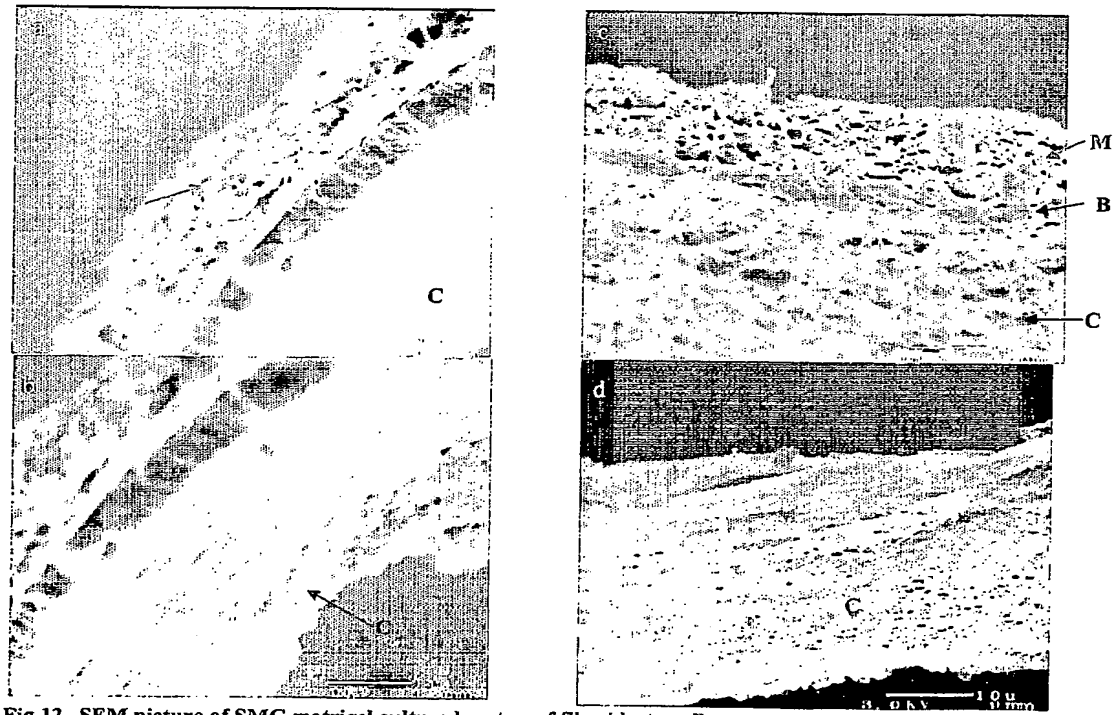

Fig 12. SEM picture of SMC-matrigel cultured on top of fibroblasts-collagen on day 0 (a and b), day 1 (c) and day 2 (d). Images of a and b are at the same location, but a was focused on the matrigel layer, while b was focused on the collagen layer. The meanings of symbols in the pictures are: M – SMC-matrigel; C – fibroblasts-collagen; B – boundaries between two layers.

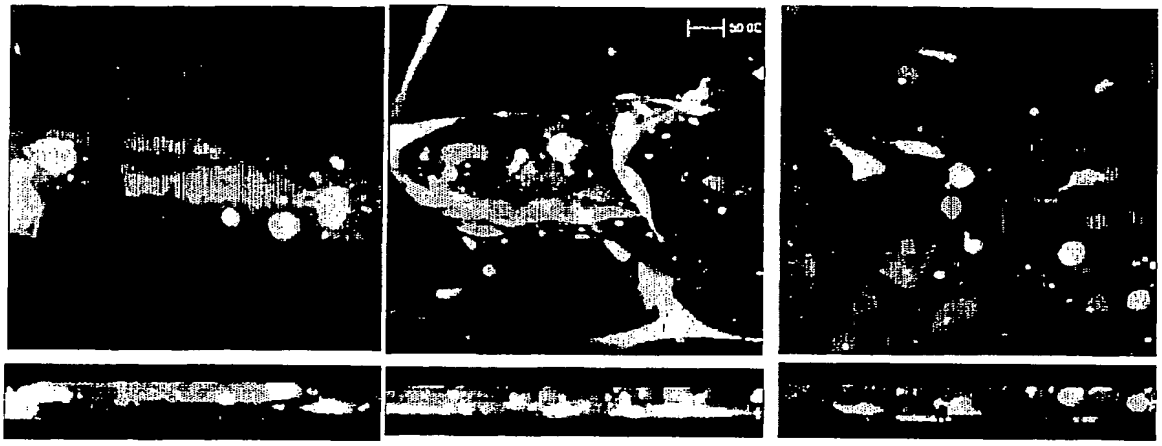

Fig 13. HUVECs migration on the layer of SMC-collagen (a), SMC-(collagen-chitosan) (b), or SMC-matrigel (c). Images are taken one day after cell seeding.

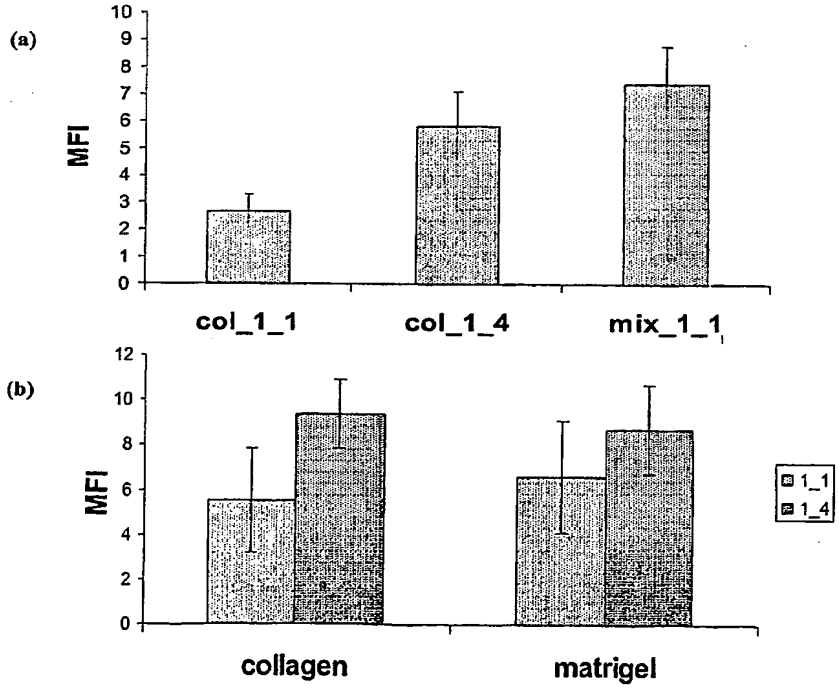

Fig 14. ICAM-1 expressions in different cocultures. (a) Microscale co-culture conditions: col_1_1 means two-layer coculture structure with 1:1 proportion of cell density between ECs and SMCs; col_1_4 with 1:4 proportion; mix_1_1 means coculture in mixed configuration; (b) Mixed co-cultures in different matrices and cell density.

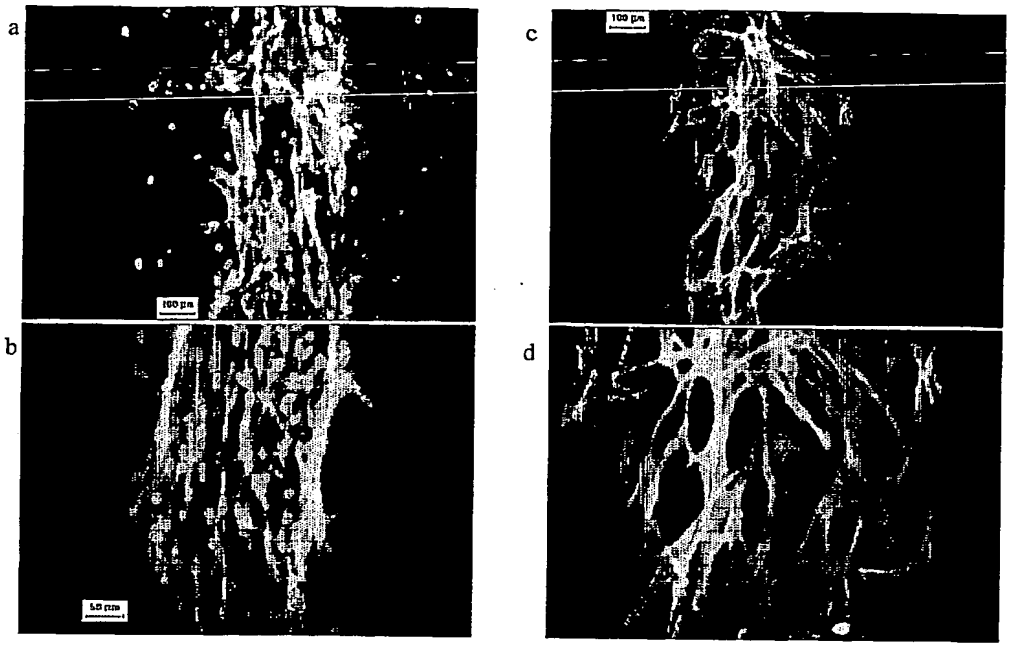

Fig 15. Cytoskeleton structure in different settings of two-layered co-cultures of HUVECs and SMCs after culturing for two days. The blue fluorescence is for the nuclei. (a-b) Co-culture in collagen matrix with 1:4 proportion of cell density between ECs and SMCs; (c-d) Co-culture in matrigel with 1:4 proportion.

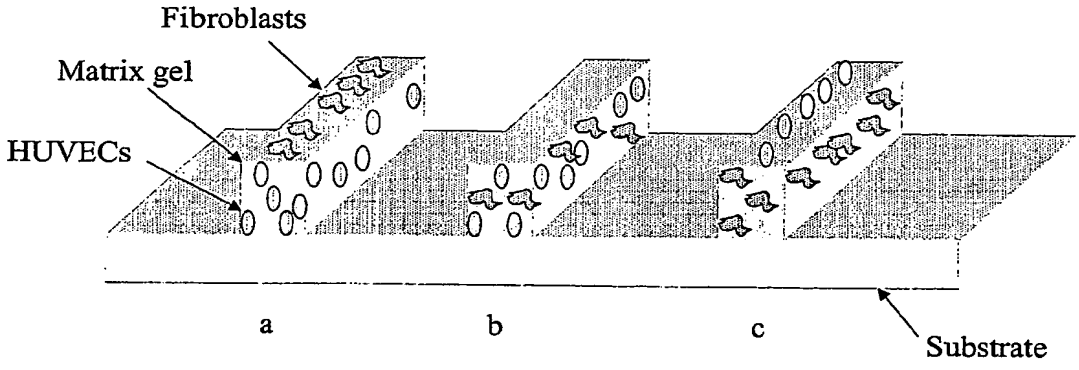

Fig 16. A schematic diagram of microstructured cell and ECM assembly. Attachment of fibroblasts on top of HUVEC-matrix microstrand. (a) Fibroblasts cultured on top of HUVECs-collagen matrix; (b) Mixed coculture of both cell types in collagen matrix; (c) HUVECs cultured on top of fibroblasts -collagen matrix.

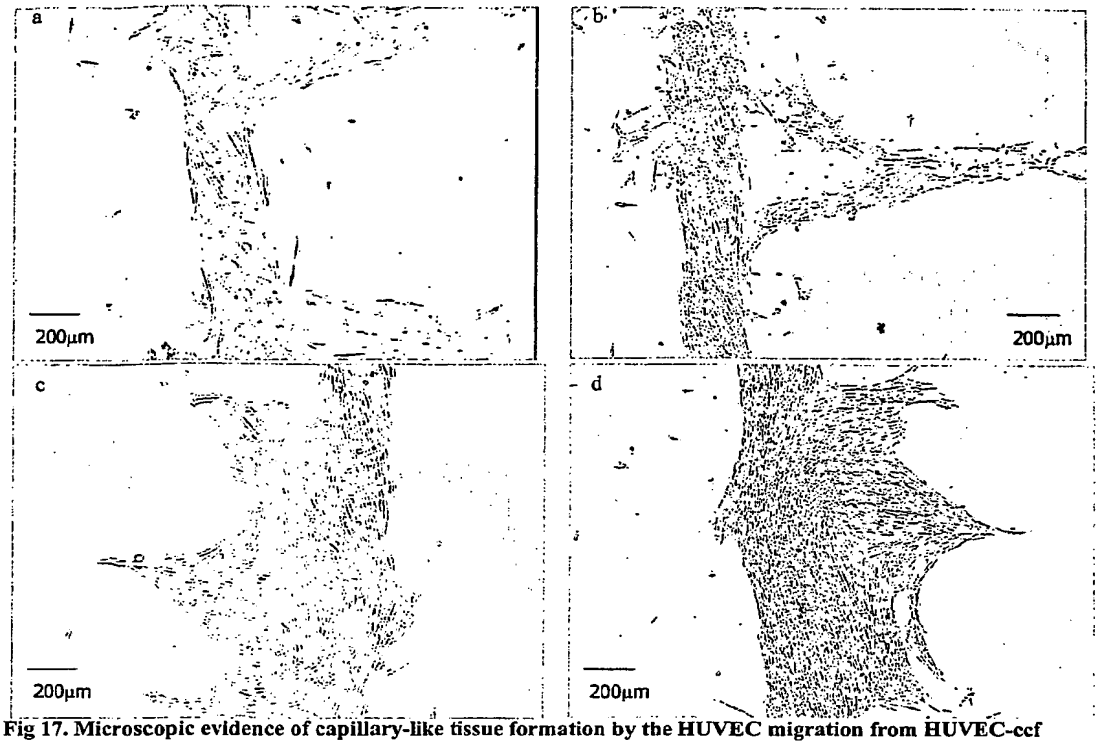

Fig 17. Microscopic evidence of capillary-like tissue formation by the HUVEC migration from HUVEC-ccf micropattern with layer of fibroblasts on top. a and b are using ccf1 (collagen: chitosan: fibronectin = 1: 1: 0.13) on day1 and day3; c and d are using ccf2 (collagen: chitosan: fibronectin = 1: 1: 0.67) on day2 and day4